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From the Armed Forces Institute of Pathology, Washington, D. C.,
Donner Laboratory, University of California, Berkeley, California,
and the National Aeronautics and Space Administration,
Ames Research Center, Moffett Field, California

DEGENERATION AND REGENERATION OF MYELINATED FIBERS IN THE CEREBRAL AND CEREBELLAR CORTEX FOLLOWING DAMAGE FROM IONIZING PARTICLE RADIATION

By Juan F. Estable-Puig,* Cornelius Tobias, (U.C.)
and Webb E. Haymaker

With 24 Figures in the Text

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Although rather numerous studies have been carried out on the effects on the cerebral and cerebellar cortex of relatively wide beams of monoenergetic accelerated particles in the medium energy range (10 to 20 Mev/nucleon), only one has been concerned with axonal degeneration and regeneration (ROSE et al., 1959, 1963), and none with the

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Dedicated to Professor Hugo Spatz on the occasion of his 75th birthday (September 2), with felicitations.

*National Institutes of Health Fellow (Post-Doctoral Research Fellowship FFG-187), AFIP, on leave of absence from Instituto de Neurología and Instituto de Patología y Medicina Experimental, Facultad de Medicina, Montevideo, Uruguay. Present Address: Ames Research Center, NASA, Moffett Field, California.

behavior of the myelin under these conditions. To help fill this gap is the purpose of this paper. In the studies with which this report is concerned, damage was incurred on the basis that as particle velocity decreases in passing through tissue the rate of energy transfer and the absorbed dose increase, so that at the ionization peak ("BRAGG peak") in the region of termination of the particle beam the damage is most intense (BAKER et al.; JANSSEN et al., MALIS et al., 1958, 1960, 1962; TOBIAS).

The initial work in this field was carried out on the cerebral cortex of 2 cats (MALIS et al., 1957). Protons having an energy of 10 Mev were used and the radiation dose at the peak of the BRAGG curve was approximately 5 000 rad. The beam diameter was not indicated, but was relatively wide. At 6 months after irradiation virtually all the nerve-cell bodies in a band ("BRAGG-peak band") in a region 100 μ in width and 800 μ deep* to the brain surface had disappeared, leaving relatively intact the pre-existing tissue framework, in which proliferated glia (type unspecified) were encountered. In accordance with the monoenergetic nature of the particles, the lower border of the band of nerve-cell loss was straight and sharp, and throughout its length was equidistant from the cyclotron aperture. The upper border of the band was, by contrast, somewhat uneven because of the irregular loss of nerve cells.

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*All the widths and depths referred to in this paper were determined on stained sections.

In subsequent studies carried out by this same group of investigators, 20-Mev deuterons, 10-Mev protons and 40-Mev alpha particles were used, but preference was given to the deuteron beam because its range in the brain (about 2.5 mm) was twice that of the other beams. The cyclotron aperture was 3 to 4.5 mm in diameter. Cats and rabbits were utilized. At deuteron surface doses of 3 000 to 9 000 rad (peak doses, 15 000 to 45 000 rad) the pathological changes were of much the same nature as observed in the initial experiment described in the foregoing. The width of the "band" varied with the radiation dose, e.g., from approximately 60 to 100 μ at surface doses of 3 000 to 5 000 rad (peak doses, 15 000 to 24 000 rad), respectively. Under these conditions destruction of nerve-cell bodies was virtually limited to the "band," while with greater "band" widths, e.g., 180 μ at 9 000 rad surface dose (37 000 rad peak dose), nerve-cell bodies in the region just above the band or even at higher levels also underwent destruction. Calculations indicated that narrow to wide bands were produced by doses of 4×10^9 to 12×10^9 deuterons/cm^2, respectively (MALIS et al., 1958, 1960, 1962).

Detailed studies on axis-cylinder destruction and regeneration in these brains were carried out by ROSE et al. (1960), whose approach was that of describing axonal regrowth after exposure to different radiation doses over various time periods, and not that of following the sequence of regrowth in point of time at any given dosage. Surface doses utilized in this study were approximately 3 000 to 9 000 rad (peak doses, 15 000 to 45 000 rad).

As to degenerative changes following irradiation, ROSE et al. (1960) reported that nerve fibers were more radiovulnerable than nerve-cell bodies. This was brought out in sections of brain exposed (a) to a surface dose of 4 000 rad (peak dose, 20 000 rad), in which, at 21 days following irradiation, the band was considerably rarefied as far as nerve fibers were concerned (SCHULTZE's silver method was employed), but contained numerous nerve-cell bodies (their Figs. 30 and 31), and (b) to a surface dose of 10 000 rad (peak dose, 40 000 rad), in which, after a 30-day period, virtually all nerve fibers in the entire irradiated area of the cortex had disappeared, while most of the nerve-cell bodies above the band were intact (their Figs. 9, 10, and 11). A further point made was that apical dendrites traversing the band were considerably less radioresponsive than axis cylinders in the self-same location. This was evident, for instance, at the 132-day stage in a brain exposed to a surface dose of 12 000 rad (peak dose, 20 000 rad) (their Fig. 33).

Axonal growth occurred after a certain time lapse (exact time not specified), as was evident by the accumulation of delicate fibers within the band. Such fibers appeared much earlier when the radiation dose was relatively low than when nearly maximal for tissue destruction. With the passage of time, the nerve sprouts in the band increased greatly in number, ramified profusely, and formed rather dense networks which ran horizontally in the band; in some preparations the fibers extended more or less vertically across the band into the superjacent, less damaged, cortex. The morphological end-point of new growth was considered to have

been reached by at least 7 weeks. At this time period the pattern of axonal growth within the band varied with the dose: at moderate radiation levels (surface dose, 6 000 rad; peak dose, 28 000 rad), restitution of the neurofibrillary pattern was achieved, while at doses nearly maximal for tissue destruction (surface dose, 9 000 rad; peak dose, 39 000 rad) the newly grown fibers were much denser and were patternless.

These various papers deal only with cerebral cortex. No detailed study on the behavior of myelinated fibers after irradiation of the cerebellar cortex is known to us.

MATERIALS AND METHODS

A total of 54 rats of the LONG-EVANS strain, 3 weeks of age and weighing approximately 60 gm, were exposed to alpha particles from the 60-inch cyclotron in Berkeley. The cyclotron aperture was 14.3 mm in diameter, allowing irradiation of most of the dorsal surface of the cerebrum and cerebellum. The energy of the alpha particles was 12 Mev per nucleon. The brain-surface dose was 6 000 rad, and in the region of maximal penetration of the particles, i.e., in the region corresponding to the BRAGG peak, the dose was approximately 30 000 rad. During exposure, the average dose rate was 1 000 rad/min to the brain surface, or approximately 10^8 particles/cm^2/sec. (Upon recalculation, the figure, 4×10^9 particles/cm^2/sec, given in previously publications (JANSSEN et al.; KLATZO, et al., 1961, 1962; WOLFE et al.) was found incorrect; in those publications the value should have been 10^8 particles/cm^2/sec.) Further irradiation details are given elsewhere (JANSSEN et al.)

After exposure, the animals were sacrificed by decapitation at periods ranging from 4 hours to 7 months. The brains were fixed in chloral hydrate (10 percent), pyridine (70 percent), or alcohol-ammonia for silver nitrate impregnation en bloc (CAJAL), in formol bromide for silver carbonate impregnation, and in formalin (10 percent) for myelin staining (SPIELMEYER and WOELKE), silver impregnation of nerve fibers (GROSS-BIELSCHOWSKY, SOTELO-CAJAL, and SCHULTZE), and polarizing microscopy. Blocks of brain prepared by the CAJAL silver nitrate method, mostly according to formula 6, were embedded in paraffin or celloidin and occasionally tuned with gold chloride after sectioning. Some brains fixed in BOUIN fluid or in formalin (10 percent) were embedded in paraffin for staining by cresyl violet or by the hematoxylin-VAN GIESON and KLÜVER-BARRERA methods. Sudan IV was used as the fat stain.

PATHOLOGICAL CHANGES IN BRAIN COMPONENTS OTHER THAN IN NERVE FIBERS

An initial change following exposure to the 6 000 rad surface dose (30 000 rad peak dose) consisted in damage of occasional nerve cells in much of the irradiated cerebral cortex, but particularly in the region of maximal penetration of the particles, which was approximately 1 mm deep to the brain surface. This was at 6 hours after irradiation. The same was true for the cerebellar granular layer, though, here, many more granule cells were altered, i.e., pyknotic. By 42 hours numerous nerve-cell bodies in the most intensely irradiated region, both in the cerebrum and cerebellum, showed evidence of necrosis, making evident

a "band" (the "BRAGG-peak" band), the lower border of which was straight and sharp while the upper border was indistinct because of irregular disappearance of nerve cells. The band of nerve-cell loss was consistently wider in the cerebral than in the cerebellar cortex (an average of 110:41 μ). Despite cellular devastation within the band, the apical dendrites of many immediately subjacent nerve cells that traversed the band appeared relatively unaltered (JANSSEN et al.). Pathological changes at the 16-day stage, as revealed in a hematoxylin-VAN GIESON preparation, are indicated in Figure 1.

Fig. 1

Neuroglia were even more radiovulnerable than nerve cells. In hematoxylin-VAN GIESON preparations, necrosis of occasional neuroglia was already evident both in the cerebrum and cerebellum at 6 hours after irradiation. In CAJAL preparations, on the other hand, the earliest change observed was at 48 hours, and consisted in an increase in the intensity of metallic impregnation. By 72 hours, astrocytic hypertrophy was noted, but after the passage of a few more hours numerous astrocytes in the band had undergone disintegration or had vanished. Astrocytes just beneath the lower border of the band became hypertrophic, with their superior processes oriented to, and extending well up into, the band. By the 6th day, astrocytes throughout much of the irradiated area had undergone disintegration (Fig. 2), and by the 16th day, few remained. Owing to capriciousness of impregnation, the relative radiovulnerability of oligodendrocytes at early stages was not established.

Fig. 2

Activation of microglia, occurring in the more intensely irradiated cortex, was first observed at the 48-hour period. In time, the band, from which nerve-cell bodies and neuroglia had largely disappeared was occupied by myriad thorny microglial cells and possibly by some oligodendroglia (HAYMAKER, 1962, 1963; JANSSEN et al.; KLATZO et al., 1961, 1962), highly similar to those illustrated in Fig. 19.

A further significant alteration in astrocytes was the appearance, within them, of PAS-positive glycogen granules. At 6 hours after irradiation the granules were noted in occasional astroglia at various levels of the irradiated cortex. By 24 hours they were abundant and were concentrated in the region both above and below the band. Neither at this time period nor subsequently were granules found within the band. The peak of glycogen accumulation was at 48 hours. At subsequent time intervals, glycogen granules had been taken up by microglia. By the 36th day, PAS-positive granules were still found, but they contained no glycogen (KLATZO et al., 1961, 1962; MIQUEL et al.; WOLFE et al.).

Considering, now, the effects of the irradiation on vessels and on vascular permeability, vascular dilatation (as brought out by the PICKWORTH-LEPEHNE technique) was evident in the irradiated region at the 48-hour period and was most pronounced in the band. Within 3 or 4 days, many vascular filling defects were noted, especially in vessels within the band. Blood-vessel status at the 18th day after irradiation is

Fig. 3 indicated in Figure 3. Virtually simultaneous with the vascular dilatation, a disturbance of vascular permeability became evident. This was brought out through the use of fluorescein-labeled serum albumin (FLA),

which was given intravenously some 24 hours prior to sacrifice. At 48 hours, numerous vessels in the irradiated cortex were surrounded by FLA droplets and, by 72 hours, the FLA, much of it in activated microglia, was widespread in the irradiated cortex, so that the entire irradiated cortex, and even down into subjacent white matter, were grossly fluorescent. "Barrier" penetration by the FLA, as observed microscopically, lasted 36 days (JANSSEN et al., KLATZO, et al., 1961).

Comment. Damage incurred by the irradiated cortex was due to a combination of factors. Degree of damage and the latent period required before damage became evident at different tissue levels coincided with the energy given off along the slopes and at the peak of the BRAGG curve (JANSSEN et al.). The chief pathogenic factor was considered to be the radiation energy per se acting on tissue elements. This was considered the sole pathogenic factor in parenchymal cell damage up to the 48th hour. Other factors, operative from 48 hours onward, consisted in circulatory disturbances and flooding of the tissue by plasma components as a result of breakdown in the blood-brain-barrier mechanism. As a consequence of these pathogenic influences much of the irradiated tissue was gradually destroyed -- so much so that after a lapse of some months tissue atrophy in the irradiated region was extreme. As the pathological process in the irradiated region underwent resolution, the capillary network gradually became re-established. As brought out in the foregoing, by the 16th day after irradiation the BRAGG-peak band had the following characteristics: nerve-cell bodies, astrocytes and,

probably, oligodendrocytes, had disappeared, leaving intact a delicate neurogliopil; the apical dendrites of subjacent nerve cells and hypertrophied processes of subjacent astrocytes extended up into the band, reactive microglia in great numbers and a few oligodendroglia occupied the band, vessel walls were still being penetrated by FLA, and the capillary tree was becoming re-established.

DEGENERATION AND REGENERATION OF NERVE FIBERS

The sequence of change occurring in axis cylinders and in myelin following exposure to the 6 000 rad surface dose (30 000 rad peak dose) of alpha particles was as follows:

4, 8 and 24 Hours Following Irradiation (3 Animals). At these time intervals no brain changes were observed.

42 and 48 Hours (3 Animals). Sections prepared by myelin staining methods showed slight tissue pallor only in the region of the band in the interhemispheric cortex. In silver preparations a full complement of normal-appearing axis cylinders was observed.

96 Hours (3 Animals). Practically all the irradiated part of the cerebral cortex in all 3 animals showed advanced patchy reduction in myelin staining. Myelin fragments were particularly numerous in the heavily-myelinated bundle of radial fibers in the interhemispheric cortex (Fig. 4). On the whole, little orientation of the myelin damage to the vascular tree was observed.

Fig. 4

As brought about by silver impregnation, axis cylinders showed no change except in silver-nitrate block-impregnation preparations (CAJAL), in which some axis cylinders traversing the band were hyperargentophilic.

8, 9, 10, 11 and 12 Days (21 Animals). Myelin loss in the irradiated area had become more uniform and the band was more clearly established (Fig. 5). That myelin was virtually non-existent in the band was verified by polarizing microscopy (Figs. 6 and 7).

Fig. 5

Figs. 6-7

Silver impregnation was carried out only on brains at the 8- and 9-day stages. Axis cylinders in the band and sometimes above the band exhibited hyperargentophilia. Only in an occasional brain was there a definite reduction in the number of axis cylinders, especially within the band.

16 Days (8 Animals). In all the brains the band was prominent, and its lower border, sharp. In general, myelin within the band had vanished (Figs. 8 and 9), as was confirmed by the lack of birefringence by polarizing microscopy (Fig. 10). Above the band in occasional brains fragmented myelin unattended by reactive cells was found, although in considerably less measure than at the 4-day stage (Fig. 4).

Figs. 8-10

In silver preparations it was evident that the axis cylinders were far less affected than the myelin. Only within the band was there conspicuous axis-cylinder rarefaction. Yet, despite the rarefaction, a fair number of axons, judged to be newly formed because of their fineness, could be made out. As revealed by the CAJAL silver nitrate method (formula 6) and by silver impregnation, these axons were of

somewhat differing diameter and were oriented more or less parallel to the brain surface. In sections stained by the SPIELMEYER and WOELKE methods and in otherspreparedfor polarizing microscopy, the axons were clearly surrounded by myelin. As in pre-existent fibers, the myelin sheath around these newly formed axons was uniform.

24 and 30 Days (6 Animals). SPIELMEYER preparations revealed generalized and uniform myelin loss in the irradiated region, except in 2 animals (AFIP Accs. 990620 and 990628) in which radial fibers extending into the interhemispheric cortex were heavily myelinated (Fig. 11). Myelin around these fibers took on a deeper hue than normally. In all 6 brains, CAJAL preparations revealed a vast number of delicate axons within the band (Figs. 12 and 13). They were most abundant in the cortex bordering the interhemispheric fissure (Fig. 13). Moreover, these new axis cylinders had become myelinated (Fig. 14) and in some brains had formed dense networks. The number of newly-formed regenerated fibers varied: They were most abundant in regions normally heavily myelinated, such as the interhemispheric cortex, and somewhat less so in areas in which myelinated fibers are normally fine (Fig. 15). From immediately beneath the band, delicate myelinated fibers could be seen bending into the band, in which they could be followed a short distance until lost in the maze of regenerated fibers. Such entering fibers were, by far, most numerous in the region of the radial bundle extending into the interhemispheric cortex. Elsewhere beneath the band, fewer fibers were seen to turn into the band. Only occasionally could fibers be traced downward into the band from the superjacent irradiated tissue.

Fig. 11

Figs. 12-14

Fig. 15

The newly formed fibers were of varying diameter, displayed a differing degree of argentophilia, and interweaved with one another. Polarized-light examination confirmed the presence of newly formed myelin (Figs. 16-18). When viewed under high magnification, birefringence around larger axons was clearly evident (Fig. 18).

Figs. 16-18

Another finding, confirming the observations in 24-day preparations (Fig. 11) as well as those at later stages, was the presence of many fully myelinated radial fibers in the interhemispheric cortex. Moreover, silver-impregnated sections revealed, within the band, many activated argentophilic cells which were oriented parallel to the persisting axis cylinders in the band. Most of these cells were microglia, but some exhibited dark cytoplasm with few processes and a clear nucleus characteristic of that of oligodendrocytes (Fig. 19).

Fig. 19

64 Days (7 Animals). At this time period, newly myelinated fibers in the band, particularly rich in the interhemispheric cortex, had become more dense. In some of the brains the newly-formed myelinated fibers predominated in the upper part of the band and even at higher levels, where they were intermixed with persisting nerve cells.

4 and 7 Months (3 Animals). Dense collections of newly-formed myelinated fibers within the band were readily demonstrable. At 4 months the fibers were of multinodal distribution within the band, and individual fibers issuing from beneath the band could readily be seen to enter into the formation of the nerve-fiber plexus in the nodal regions (Figs. 20 and 21). In KLÜVER-BARRERA preparations the myelin

Figs. 20-21

tubes were, as at previous stages, of differing diameter. As previously, the band was densest in regions in which the fibers were richest in myelin; this difference was, however, not as striking as at earlier time intervals. Myelinated fibers were also present in the irradiated cortex above the band, but they were few and their architecture was distorted by general tissue shrinkage. By 7 months
Fig. 22 the neostria was fairly uniform (Fig. 22).

Cerebellum. Changes highly similar to those occurring in the cerebral cortex were also visible in the cerebellar cortex. Demyelination had also occurred in the irradiated crests of the cerebellar
Fig. 23 folia and was advanced by the 11th day (Fig. 23). At the 7-month stage, fibers within the irradiated part of the folia were well myelinated, and a sizable group of regenerated fibers had extended horizontally, deep into the granular layer. The neostria thus gave this region a "Lorain cross" appearance, instead of the normal Y, T or inverted L
Fig. 24 pattern (Fig. 24).

DISCUSSION

The Degenerative Process. Decrease in myelin stainability in the entire irradiated region of these brains was well advanced at a time at which axis cylinders and nerve-cell bodies were relatively intact, namely, at 96 hours following irradiation. By this time period, astrocytes in much of the irradiated area had suffered heavily, circulatory disturbances were advanced, and flooding of all irradiated tissue by plasmatic fluid was in progress. It is likely that the myelin

disruption was due in greatest measure to direct irradiation. Such damage might possibly have been brought about through a process of ionization of myelin lipoproteins, whereby their physical properties were so altered that they were no longer birefringent and their chemical properties so changed that normal staining affinities were lost. The opinion that the myelin was directly damaged by radiation is based on the rapidity of the myelin alteration and its scope and on the fact that the region of myelin involvement coincided with the field irradiated and was most profound in the lower part of the irradiated cortex, where energy loss by the particles was greatest. That edema fluid may have contributed to the myelin breakdown was indicated by the patchiness in the "myelin picture" and orientation of the demyelinative process to some vessels in early stages following irradiation. Whether damage to the oligodendrocytes was also a factor is beyond consideration because these cells could not be satisfactorily impregnated up to the 96-hour stage, when myelin damage was well underway, and whether astroglial destruction was instrumental in the demyelinative process is also not known, although it was ascertained that astrocytes seemed relatively intact in upper cortical regions from which myelin had partially vanished.

Our data indicated that axis cylinders were much less damaged than myelin. This coincides with observations by ZEMAN et al., in the brains of mice exposed to 11 Mev/nucleon deuterons at 14 000 rad in a field 1 mm in diameter (at 14 days) and at 36 000 rad in a 0.250 mm field (at 24 days). Dose rates ranging from 12.5 to 900 000 rad/sec were used, but these investigators concluded that dose rate appeared

to have little influence in the production of lesions, at least within the range they studied. On the other hand, as brought out in a previous part of this paper, studies by ROSE et al. (1960) indicated that nerve fibers were much more radioresponsive than nerve-cell bodies. (Studies on the radioresponsiveness of myelin were not carried out.) They dealt with 20-Mev (10 Mev/nucleon) deuterons at brain surface doses of 4 000 and 10 000 rad (peak doses, 20 000 and 40 000 rad), and the total number of deuterons delivered to the brain was 5.6×10^9 and 10.9×10^9 per cm^2, respectively. In our experiments, 48-Mev (12 Mev/nucleon) alpha particles at a brain-surface dose of 6 000 rad (peak dose, 30 000 rad) were used and the total number of particles delivered was 2×10^9 per cm^2. Dose rate in the ROSE et al. (1960) experiments was such that the desired dose was delivered in 1 to 2 min (MALIS et al., 1960), while in our experiments the dose rate was 10^8 particles cm^2/sec. The reason for the differing response by nerve fibers versus nerve-cell bodies in the experiments of ROSE et al. and in ours is not evident, and even conjecture is out of place because of the lack of precise physical data on energy absorption in the tissue.

In the brains in our series, spherical macrophages (gitter cells) were virtually absent until the cortex had become shrunken. Fat stains were uniformly negative. This lack of free fat in reactive microglia is in agreement with the experience of others who have been

concerned with reactions in the central nervous system following particle irradiation, including conditions under which tissue destruction occurred (LARSSON et al.; REXED et al.)

The Regenerative Process. The BRAGG-peak band in which nerve sprouts were found in our animals from approximately the 16th day onwards was characterized at that time period by a rather loose framework composed of naked axis cylinders, abundant reactive microglia and sparse oligodendroglia, and a rarefied capillary bed which was undergoing restitution. Extending up into the band from subjacent non-irradiated regions were robust processes of astroglia and the apical dendrites of nerve cells. This was the matrix, then, that was receptive to axonal regrowth. Much the same conditions were evident in the band as described by ROSE et al. (1960).

One of the aspects of the regenerative process in the brains of our animals was the remyelination of axis cylinders that had been rendered "naked" by the irradiation. As has been pointed out, many pre-existing axis cylinders traversing the band were devoid of myelin at earlier post-radiation stages (Figs. 7 and 10), while at subsequent stages the fibers in the self-same regions were myelinated (Fig. 11). This was true not only for the cerebrum but also for the cerebellum (Figs. 23 and 24). The capacity for remyelination of axis cylinders deprived of their myelin has, in the past, been considered unlikely in the central nervous system, but recent studies by BUNGE et al. have proved its occurrence in the long spinal tracts in the cat. These investigators produced plaques of demyelination subpially in

the spinal cord by repeatedly withdrawing and re-injecting cerebrospinal fluid into the spinal subarachnoid space. The axis cylinders were completely demyelinated by 6 days. Subsequently, occasional myelin sheaths were first seen at 19 days, and by 64 days all axis cylinders were at least thinly myelinated. The mechanism of myelin formation was characteristic of that observed during normal development and was basically similar to that occurring in peripheral nerves.

The other aspect of the regenerative process was the development of a robust bundle of newly-formed fibers within the BRAGG-peak band. Apical dendrites coursing upward into the band both in our preparations and in those of ROSE et al. have been considered capable of giving off sprouts under a variety of pathological conditions, but such sprouting has been found rare and evanescent (CAJAL; LAFORA). In our preparations, virtually all nerve sprouts within the band issued from the immediately subjacent tissue, namely from cortical afferent fibers. As brought out by various stains and by polarizing microscopy, myelinated fibers accumulated in due time in the band and formed a conspicuous myelinated "neostria." They grew without reconstituting the previous nerve-fiber architecture, as observed by ROSE et al. in brains exposed to the higher doses of deuterons. Up through the 4-month stage, the neostria tended to have a multinodal character (Fig. 21). It seemed that fibers entering the band accumulated in nodal regions of the band, then grew horizontally within the confines of the band until, at the 7-month stage (Fig. 22), the band was uniformly populated. Thus, it seemed that fiber growth in

the brains of our animals continued well beyond the 7-week stage, the time at which the morphological end-point of new growth was considered by ROSE et al. (1960) to have been reached in their animals.

As to the mechanism of myelin regeneration, we have little to offer. As shown in Figure 19 and as has been brought out previously (KLATZO et al., 1961; JANSSEN et al.), reactive microglia came to occupy the band in vast numbers. Astrocytes beneath the band became strikingly hypertrophic and sent their processes up into the band, where they were oriented vertical to the developing myelinated neofibers. Whether the few reactive oligodendroglia present contributed to the myelination is not known.

In drawing on a voluminous literature, ROSE et al. (1960) have given the pros and cons as to whether the axonal sprouting represents a regenerative process following severance of axons -- i.e., was provoked as a reaction to the injury that had been inflicted -- or whether the new growth was an expression of an innate capacity possessed by the central nervous system for continual axonal growth as a reconstitutive process when conditions favorable to the reconstitution exist. Mainly on the basis of restoration of nerve-fiber pattern in the damaged laminae at the lower deuteron dosage they utilized, ROSE et al. (1960) favored the latter alternative. We have nothing to add in this connection. In our preparations the richness of the regenerative process impressed us, as did also its constancy. There must have been some unusual factor or factors to account for this, for under other experimental conditions the

regrowth of fibers is usually not as luxuriant or as constant. Obviously the setting was unusually conducive to normal growth in the band, i.e., rarefied tissue in which to grow, the presence of apical dendrites and astroglial processes to guide the fibers into the band, and lack of astroglial and connective-tissue scar to impede growth. Many axis cylinders were damaged or severed in areas of irradiated cortex above the band, but the lack of at least obvious fiber regrowth may be laid to a setting less conducive than the band to the regrowth.

SUMMARY

This study was concerned with the deleterious effects of 48-Mev alpha-particle radiation, at a 6 000 rad surface dose, on nerve fibers and myelin sheaths of the cerebral and cerebellar cortex and with subsequent nerve-fiber regeneration.

Striking demyelination in the irradiated part of the cortex was already evident at the 96-hour period, but was most prominent in the region of maximal energy release, i.e., in the "BRAGG-peak band." In time, the demyelination became still more profound. The myelin destruction was attributable chiefly to direct tissue irradiation and in much less measure to an edematous process. Axis cylinders were far less radiovulnerable than the myelin, but those in the band were considerably rarefied.

Around the 16th day after irradiation, a regenerative process was under way. Evidence was obtained that pre-existing axis cylinders that had lost their myelin became remyelinated. Myelinated nerve sprouts

issuing chiefly from afferent fibers beneath the band began populating the band at this time period and grew progressively within it. The pre-existing cortical fiber-architecture was not restored. The end-stage of fiber regrowth was considered to be between the 4th and 7th month after irradiation.

The new growth was considered an expression of an innate capacity possessed by the central nervous system for continual axonal regrowth. A factor conducive to the luxuriant and constant regrowth under the irradiation conditions cited may have been the setting, namely, the rarefied nature of the tissue into which the sprouts grew, the presence of apical dendrites and astroglial processes to guide the growing fibers, and the lack, in the band, of astroglial or connective-tissue scar to restrict growth.

Only reactive microglia were present in abundance in the region of axonal regrowth. Whether oligodendroglia had any influence on the remyelination cannot be stated.

Similar changes were found to occur in cerebellar cortex under the same experimental conditions.

REFERENCES

BAKER, C. P., H. J. CURTIS and W. ZEMAN: The design and calibration of a deuteron microbeam for biological studies. Radiation Res. 15 489-495 (1961).

BUNGE, M. B., R. P. BUNGE and V. H. RIS: Ultra-structural study of remyelination in an experimental lesion in an adult cat spinal cord. J. Biophys. Biochem. Cytol. 10, 67-94 (1961).

CAJAL, RAMÓN y, S.: Degeneration and regeneration of the nervous system. Oxford: Oxford Univ. Press 1928, pp.

HAYMAKER, W.: Morphological changes in the nervous system following exposure to ionizing radiation. In: Effects of Ionizing Radiation on the Nervous System, pp. 309-358. Vienna: IAEA 1962.

HAYMAKER, W.: Myelin vulnerability, nerve-fiber regeneration and glycogen increase in the rat brain following exposure to alpha-particle radiation. With effects also of X-radiation on brain glycogen. Radiation Res. In press, 1963

JANSSEN, P., I. KLATZO, J. MIQUEL, T. BRUSTAD, A. BEHAR, W. HAYMAKER, J. LYMAN, J. HENRY and C. TOBIAS: Pathologic changes in the brain from exposure to alpha particles from a 60-inch cyclotron. In: Response of the Nervous System to Ionizing Radiation (T. J. HALEY and R. S. SNIDER, eds), pp. 383-409. New York: Academic Press 1962.

KLATZO, I., J. MIQUEL, W. HAYMAKER, C. TOBIAS and L. S. WOLFE: Observations on appearance of histochemically-demonstrable glycogen in the rat brain as effect of alpha-particle irradiation. In: Effects of Ionizing Radiation on the Nervous System, pp. 309-358. Vienna: IAEA 1962.

KLATZO, I., J. MIQUEL, C. TOBIAS and W. HAYMAKER: Effects of alpha-particle radiation on the rat brain, including vascular permeability and glycogen studies. J. Neuropath. exp. Neurol. 20, 459-483 (1961).

LAFORA, G. R.: Neoformaciones dendriticas en las neuronas y alteraciones de la neuroglia en el perro senil. Trab. Lab. Invest. Biol. 12, 39-53 (1914).

LARSSON, B., L. LEKSELL, B. REXED and P. SOURANDER: Effect of high energy protons on the spinal cord. Acta radiol. (Stockh.) 51, 52-64 (1959).

MALIS, L. I., C. P. BAKER, L. KRUGER and J. E. ROSE: Effects of heavy, ionizing, monoenergetic particles on the cerebral cortex. I. Production of laminar lesions and dosimetric considerations. J. Comp. Neurol. 115, 219-242 (1960).

MALIS, L. I., L. KRUGER and J. E. ROSE: The use of deuterons in production of laminar lesions in the cerebral cortex of the rabbit. Trans. Am. Neurol. Assn. 83, 78-80 (1958).

MALIS, L. I., R. LOEVINGER, L. KRUGER and J. E. ROSE: Production of laminar lesions in the cerebral cortex by heavy, ionizing particles. Science 126, 302-303 (1957).

MALIS, L. I., J. E. ROSE, L. KRUGER and C. P. BAKER: Production of laminar lesions in the cerebral cortex by deuteron irradiation. In: Response of the Nervous System to Ionizing Radiation (T. J. HALEY and R. S. SNIDER, eds), pp. 359-368. New York: Academic Press 1962.

MIQUEL, J., I. KLATZO, D. B. MENZEL and W. HAYMAKER: Glycogen changes in X-irradiated rat brain. Acta neuropath. In Press, 1963.

REXED, B., W. MAIR, P. SOURANDER, B. LARSSON and L. LEKSELL: Effect of high energy protons in the brain of the rabbit. Acta radiol. (Stockh.) 53, 289-299 (1960).

ROSE, J. E., L. I. MALIS and C. P. BAKER: Neural growth in the cerebral cortex after lesions produced by monoenergetic deuterons. International Symposium on Principles of Sensory Communication, 1959. In Press, 1963.

ROSE, J. E. L. I. MALIS, L. KRUGER and C. P. BAKER: Effects of heavy, ionizing, monoenergetic particles on the cerebral cortex. II. Histological appearance of laminar lesions and growth of nerve fibers after laminar destructions. J. Comp. Neurol. 115, 243-296 (1960).

TOBIAS, C. A.: The use of accelerated heavy particles for production of radiolesions and stimulation of the central nervous system. In: Response of the Nervous System to Ionizing Radiation (T. J. HALEY and R. S. SNIDER, eds), pp. 325-343. New York: Academic Press 1962.

WOLFE, L. S., I. KLATZO, J. MIQUEL, C. TOBIAS and W. HAYMAKER: Effect of alpha-particle irradiation on brain glycogen in the rat. J. Neurochem. 9, 213-218 (1962).

ZEMAN, W., H. J. CURTIS and C. P. BAKER: Histopathologic effect of high-energy-particle microbeams on the visual cortex of the mouse brain. Radiation Res. 15, 496-514 (1961).

FIGURE LEGENDS

Fig. 1 Cerebral cortex. 16 days after irradiation. The "Bragg-peak band," from which most of the nerve cells have vanished, is clearly evident. X 50. Hematoxylin-VAN GIESON stain. (AFIP Acc. 961739; Neg.).

Fig. 2. Cerebrum. 6 days after irradiation. The "Bragg-peak band" (BPB) is virtually free from astrocytes, but some nerve cells persist. Above the band the astrocytes are in a degenerated state. Just below the band, astroglia have proliferated, and their processes are oriented to the band. X 5.4. CAJAL gold sublimate method. (AFIP Acc. 517618; Neg. 61-564).

Fig. 3. Cerebral cortex. 18 days after irradiation. The "Bragg-peak band" (BPB) is recognizable as a pale strip. The larger blood vessels passing through the band and in the overlying cortex are characterized by irregular varicose dilatations. X 90. PICKWORTH-LEPEHNE stain. (AFIP Acc. 517629) (From KLATZO et al., 1961).

Fig. 4. Cerebral cortex. 96 hours after irradiation. Patchy but striking myelin loss is to be noted through much of the irradiated cortex, marked off inferiorly by a rather indistinct line. There is little relation of the demyelination to the vascular tree. X 50. SPIELMEYER myelin stain. (AFIP Acc. 990658; Neg. 61-5836).

Fig. 5. Interhemispheric occipital cortex. 8 days after irradiation. Intense demyelination of the deeper part of the irradiated area has occurred. A sharp border between irradiated and non-irradiated tissue is to be noted. Vessels are engorged with blood. X 50. Frozen section. SPIELMEYER myelin stain. (AFIP Acc. 990633; Neg. 61-4840).

Figs. 6 and 7. Cerebral cortex. 9 days after irradiation. Fig. 6. A myelin-free band is to be seen midway down the photograph, at the level of maximal ionization ("BRAGG-peak band"). Fig. 7. From the same section, in which the specimen was rotated 90°, illustrating the absence of birefringement material in the "BRAGG-peak band." X 30. Frozen section. Polarized-light photomicrographs. (AFIP Acc. 951521).

Figs. 8-10. Cerebral cortex. 16 days after irradiation. Fig. 8. Sharp interruption is to be noted in the striae of BAILLARGER (sB) and the radial fibers (rf) at the level of the peak of ionization. X 50. Fig. 9. Lateral region of visual cortex. Myelin loss is to be noted in the entire irradiated area, with greatest loss in the region of peak ionization. X 50. Figs. 8 and 9. Frozen sections. SPIELMEYER myelin stain. Fig. 10. Lateral region of visual cortex. Birefringency has disappeared from the irradiated region, marked off from the non-irradiated tissue by a sharp line. X 30. Frozen section. Polarized-light photomicrograph. (AFIP Accs. 990621, 961739,and 951523; Negs. for Figs. 8 and 9, 61-4838 and 61-4827).

Fig. 11. Cerebral cortex. 24 days after irradiation. Extensive and uniform myelin loss has occurred except for the radial fibers, which are only partially demyelinated. A faint "BRAGG-peak band" (BPB) is noticeable. X 50. Frozen section. SPIELMEYER myelin stain. (AFIP Acc. 990620; Neg. 61-4837).

Fig. 12-14. Cerebral cortex. 30 days after irradiation. Fig. 12. Newly grown axons run horizontally in the "BRAGG-peak band." Axis cylinders are much more numerous below than above the band. X 300. Fig. 13. 30 days after irradiation. Axonal regrowth is prolific in the region of the interhemispheric fissure (arrow). X 50. CAJAL silver-nitrate impregnation. Fig. 14. 24 days after irradiation. Delicate myelinated fibers are to be seen in the "band" (identified by lines). X 115. Frozen section. SPIELMEYER myelin stain. (AFIP Accs. 951526, 951526 and 990620; Negs. 61-4833, 62-1604 and 61-4836).

Fig. 15. Cerebral cortex. 30 days after irradiation. A bundle of newly formed myelinated fibers stretches across the cortex, in the upper part of the "BRAGG-peak band." Numbers of fibers vary in the different regions. X 80. Frozen section. SPIELMEYER myelin stain. (AFIP Acc. 951527; Neg. 613212).

Fig. 16-18. Cerebral cortex. 30 days after irradiation. Under polarized light no evidence of myelin is to be seen (Fig. 16), but, as revealed by birefringence, myelinated fibers do become evident on 90° rotation of the specimen (Figs. 17 and 18). Fig. 18 is an enlargement of a field indicated in Fig. 17. Frozen sections. Polarized light photomicrographs. (AFIP Acc. 990635).

Fig. 19. Cerebral cortex at level of "BRAGG-peak band." 30 days after irradiation. The arrows indicate the principal cellular components, microglia (M) and oligodendroglia (O). X 300. Frozen section. HORTEGA silver carbonate impregnation. (AFIP Acc. 990635; Neg. 612203).

Figs. 20 and 21. Cerebral cortex. 4 months after irradiation.

Fig. 20. Visual cortex. Prominent, multinodal myelinated fiber bundles extend across the cortex in the upper part of the "BRAGG-peak band." Groups of myelinated fibers from lower laminae appear to be entering these nodal points. X 50. A field in Fig. 20 is illustrated in higher magnification in Fig. 21. Here, individual fibers can be seen extending into the nodal fiber aggregates in the band. X 305. Celloidin section. WOELKE myelin stain. (AFIP Acc. 951529; Negs. 61-4661 and 61-4660).

Fig. 22. Region of interhemispheric cerebral cortex. 7 months after irradiation. The irradiated region of the cortex is severely atrophic. A dense, myelinated bundle continuous with the subcortical white matter occupies the "BRAGG-peak band." X 50. Paraffin section. Luxol Fast Blue stain. (AFIP Acc. 943707; Neg. 61-4666).

Figs. 23 and 24. Cerebellum. Fig. 23. 11 days after irradiation. The area irradiated is marked off inferiorly by a sharp border. Striking loss of myelin is to be noted in the lowermost irradiated region ("BRAGG-peak band"), and above this level relatively few myelinated fibers are to be seen. PURKINJE cells in the lower part of the irradiated area have vanished, while many of those remaining are hyperchromatic. X 5. Paraffin section. KLÜVER-BARRERA stain. Fig. 24. 7 months after irradiation. The irradiated part of the folia is severely atrophic. Myelinated fibers in this region have become reconstituted, and a broad sheaf of myelinated neofibers in the region of the "BRAGG-peak band" extends from the white-matter core laterally into the granular layer, reaching the molecular layer. A configuration simulating a Loraine cross has thus been created. X 50. Paraffin section. Luxol Fast Blue stain. (AFIP Accs. 961735 and 943707; Negs. 61-6458 and 61-4669).

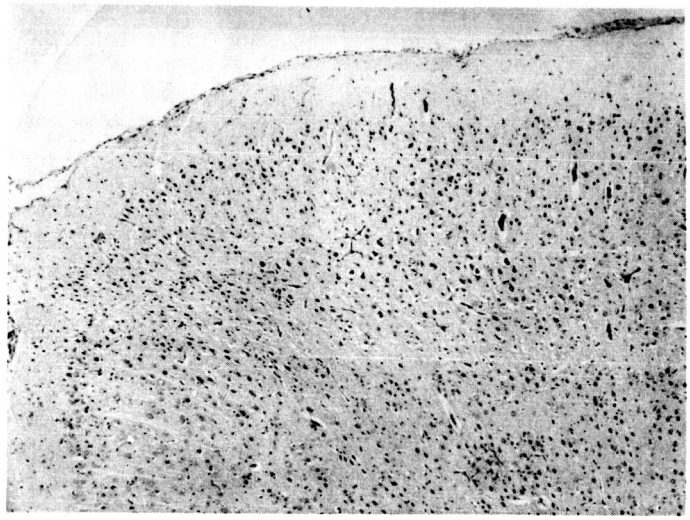

Figure 1.

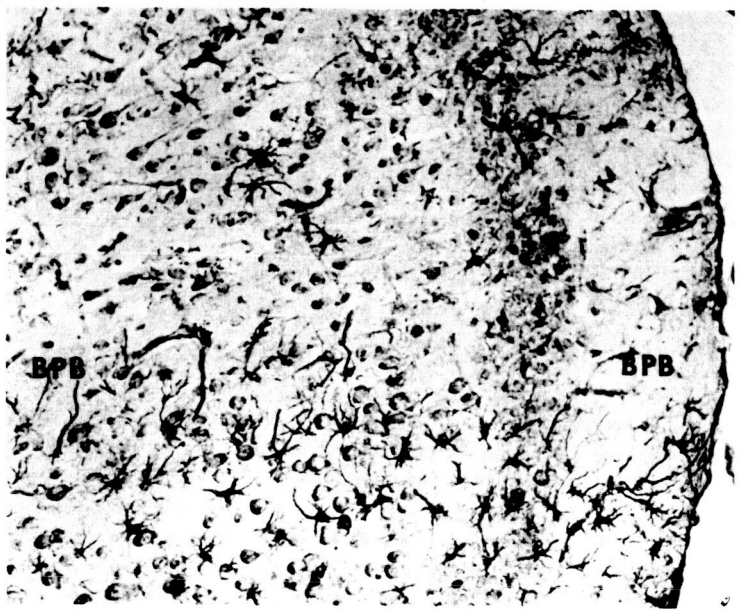

Figure 2.

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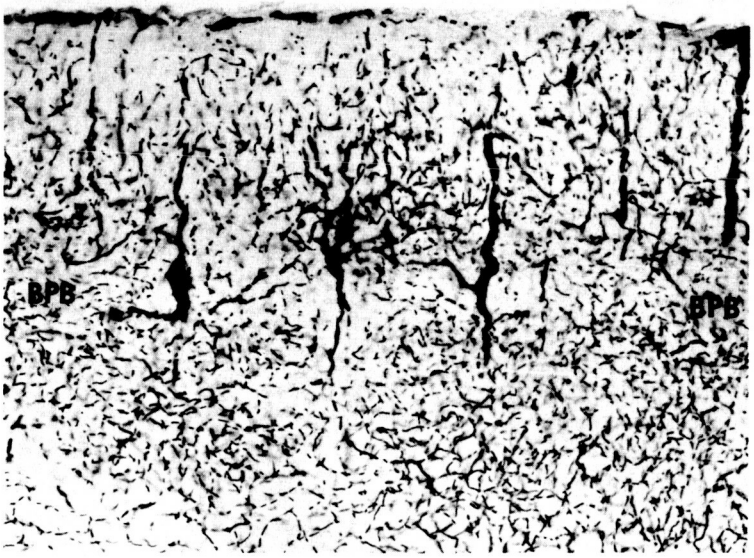

Figure 3.

Figure 4.

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Figure 5.

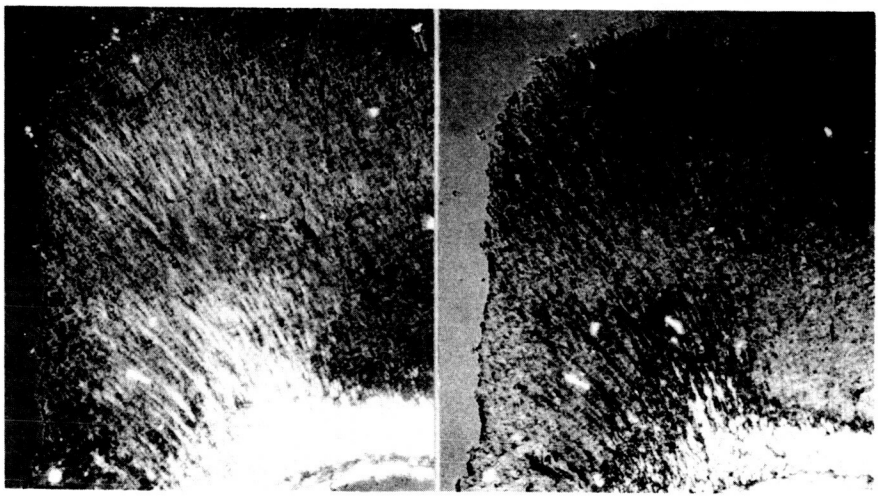


Figure 6-7.

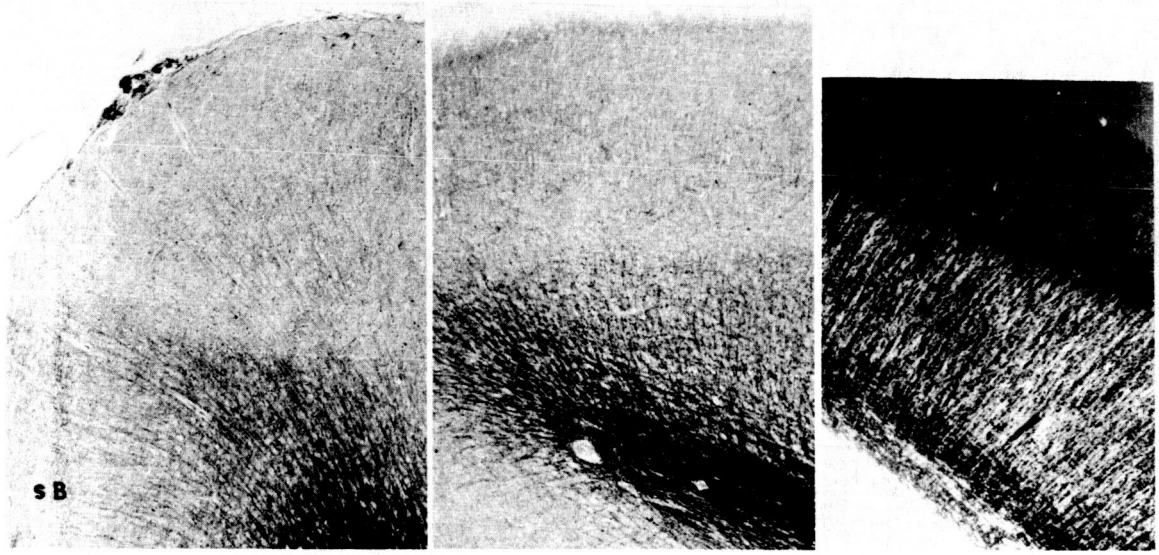
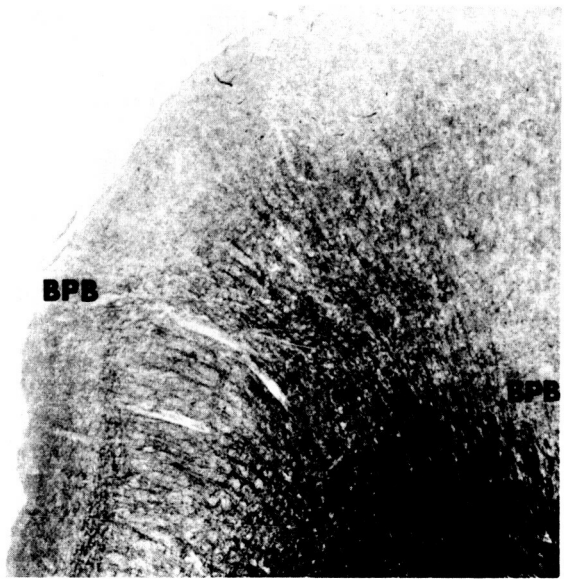


Figure 8-10.



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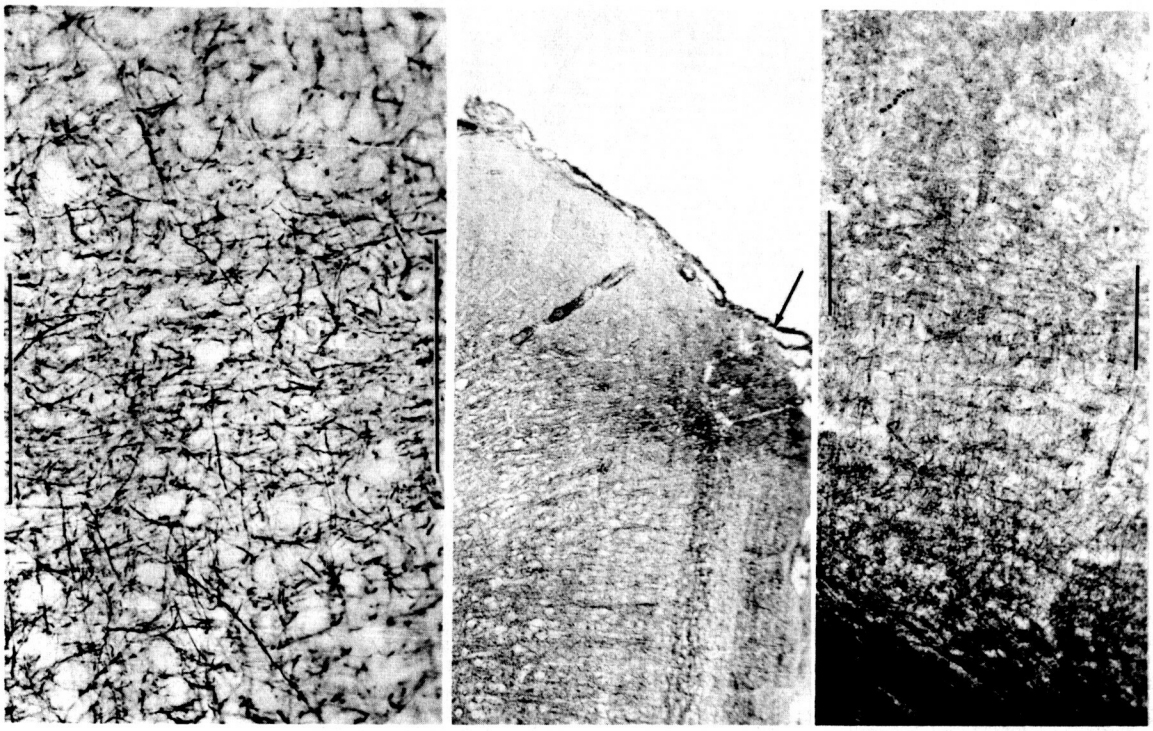
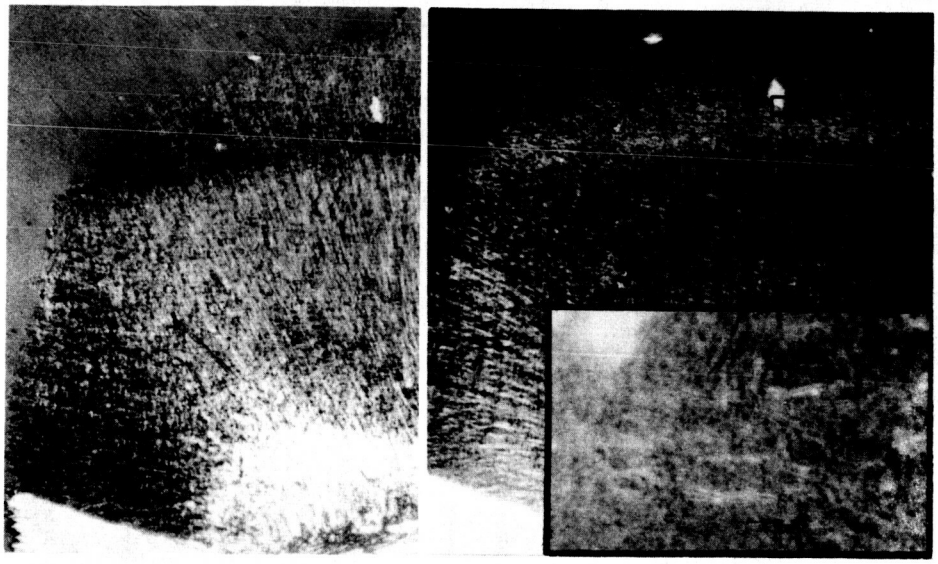


Figure 12-14.

Figure 15.



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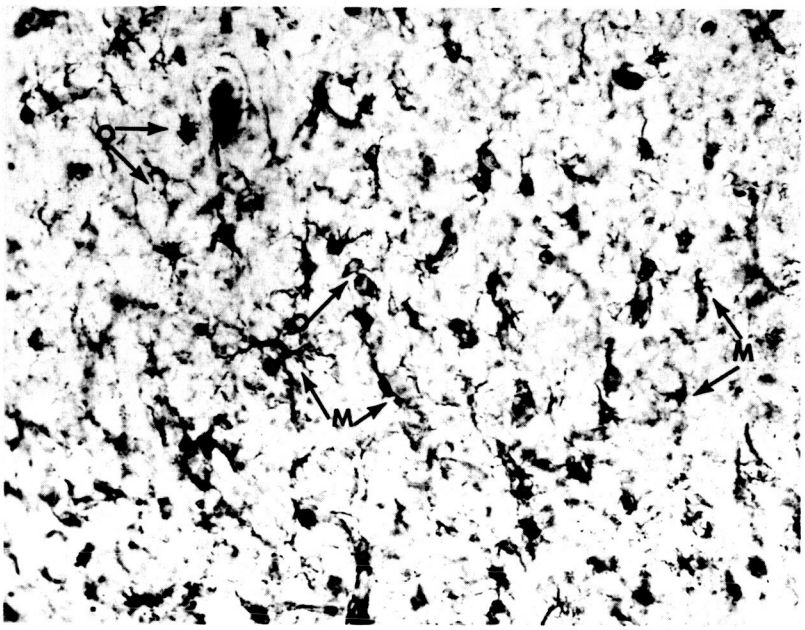

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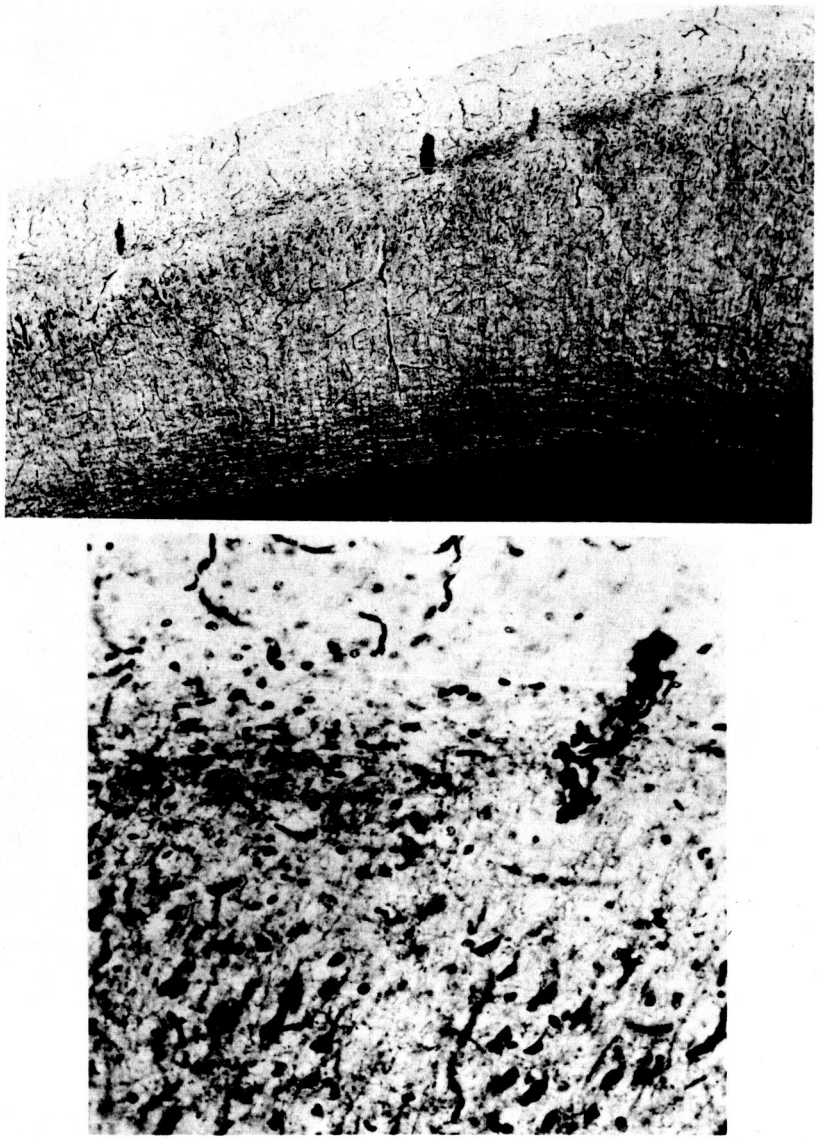

Figure 20-21.

Figure 22.

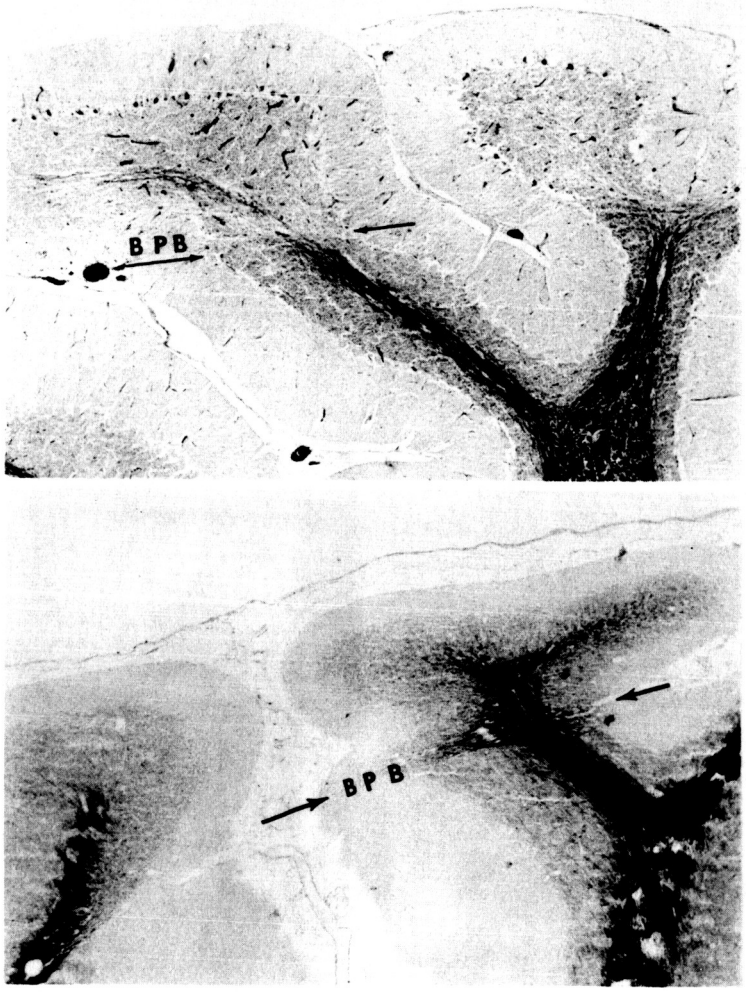

Figure 23-24.